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The Effects of Fructose on Contractility of Isolated Rat Atria Depressed with Lidocaine

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ABSTRACT

The effect of metabolic substrate fructose on the force of contraction of isolated rat atria depressed with lidocaine was determined. Fructose produced dose-dependent increase in the force of contraction of isolated atria depressed by substrate-free Krebs-Ringer bicarbonate medium. The maximally effective concentration of fructose was 30 mM.

The isolated atria, suspended in Krebs-Ringer bicarbonate glucose medium aerated with 95% O_2-5% CO_2 at 30°C and pH 7.4, were depressed 50% by approximately 2.34 mg/100 ml of lidocaine.

Addition of 30 mM fructose to these depressed atria resulted in a marked increase in the contractile force similar to that with pyruvate and acetate. Fructose had no significant effect, however, on atria exposed to low-calcium medium.

The results are consistent with a previous report suggesting blockade by lidocaine of the uptake or utilization of glucose in the glycolytic pathway, and further pinpoint the blockade as an early step in the glycolytic sequence prior to the phospho-fructokinase step.

Key Words: Lidocaine, Heart, Fructose, Contractility, Glycolysis

INTRODUCTION

It has been postulated that the cardiac depressant action of inhalation anesthetics halothane or methoxyflurane is at least partly linked to a block at an early step or steps in the glycolytic pathway in the heart of rat atria, as shown by the abilities of pyruvate, lactate, acetate, and fructose, but not glucose, to produce a positive inotropic effect in rat atria depressed by the general anesthetics (Paradise and Griffith, 1965; Ko and Paradise, 1969a; Paradise and Ko, 1970; Ko and Paradise, 1971b; 1972a; 1972b; 1973; 1975). Substrate studies in isolated human atria also suggest a block in glycolysis by these anesthetics (Ko and Paradise, 1970a; 1970b; Krishna and Paradise, 1972).

Biochemical support for this theory was demonstrated in isolated rat atria (Morrow and Paradise, 1972; 1973). Functional studies further point to the glucose phosphate isomerase step as the step inhibited by these anesthetics (Ko and Paradise, 1971a; 1971c).

It is evident that local anesthetic lidocaine also depress the cardiac function (Austen and Moran, 1965; Contantino *et al.*, 1969; De Jong *et al.*, 1973; Kahn *et al.*, 1982; Liu *et al.*, 1982; Sage *et al.*, 1983). It has been reported that pyruvate and acetate partially restored the contractility of isolated rat atria depressed approximately 50% with lidocaine, despite the fact that additional glucose had no significant

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effect on the depressed contractility of atria by lidocaine (Lim and Kim, 1984).

Present studies represent an attempt to localize further the site of lidocaine action in the glycolytic sequence of rat atria by using the metabolizable substrate, fructose.

MATERIALS AND METHODS

Male rats weighing 180 to 200g. were decapitated, and the atria were removed and suspended in modified Krebs-Ringer bicarbonate glucose medium (Gimeno *et al.*, 1965, 1966).

The medium was gassed with 95% O_2-5% CO_2 at pH 7.4 and 30°C. The mechanical activity of rat atria electrically stimulated at a rate of 200 per minute in the medium was determined using a sensitive strain gage as previously described (Gimeno *et al.*, 1966; Ko and Paradise, 1973a).

In the experimetns with substrate-free medium, the normal Krebs-Ringer bicarbonate glucose medium was changed to substrate-free medium (free of glucose) following the one-hour equilibration period.

In the experiments with low-calcium medium, the medium was prepared by omitting a 1/2 or 3/4 amount of calcium chloride from the Krebs-Ringer bicarbonate glucose medium. For the experiments, the normal Krebs-Ringer bicarbonate glucose medium was changed to this low-calcium medium following the one-hour equilibration period.

RESULTS

Effect of substrates on atria depressed with lidocaine

It has been previously demonstrated that lidocaine produce dose-dependent decrease in the force of contraction of isolated rat atria (Lim and Kim, 1965). It is evident in Fig. 1 that the lidocaine at a concentration of 0.1 mM produced approximately 50% depression in the contractile activity of atria at 30 minutes after the administration of lidocaine, and the declined degree of atrial contractility was almost kept constant levels during a 60 minutes of experimental period.

Substrates were added to the bathing medium 30 minutes after the atria were depressed about 50% with 0.1 mM of lidocaine. It is also evident from the Fig. 1 that pyruvate and acetate partially restored the contractility of atria depressed by lidocaine, but glucose was without effect.

Effect of fructose on substrate-depleted atria

The effect of fructose on the functional properties of atria had to be established to study its action on lidocaine-depressed atria. Experiments were designed using substrate depleted medium to determine the contractile behavior of atria in the absence of exogenous substrate, to provide control data with which the response to fructose might be compared.

Results are summarized in Fig. 2. Developed tension of the atria progressively decreased in the substrate-free medium after the one-hour equilibration period with Krebs-Ringer bicarbonate medium.

After 30 minutes in the substrate-free medium, fructose was added at a concentration of 10, 20, and 30 mM. It is evident from the Fig. 2 that the addition of fructose resulted in marked recovery of the force of contraction of substrate-depleted atria, and the maximally effective concentration of fructose was 30 mM. The same concentration of the nonmetabolized sugar, sucrose, however, had no effect on the substrate-depleted atria, indicating that the action of fructose at this high concentration is as result of its metabolism (Paradise and Ko, 1970).

Effect of fructose on atria depressed with lidocaine

Addition of 30 mM fructose 30 minutes after administration of lidocaine (0.1 mM) resulted in a pro-

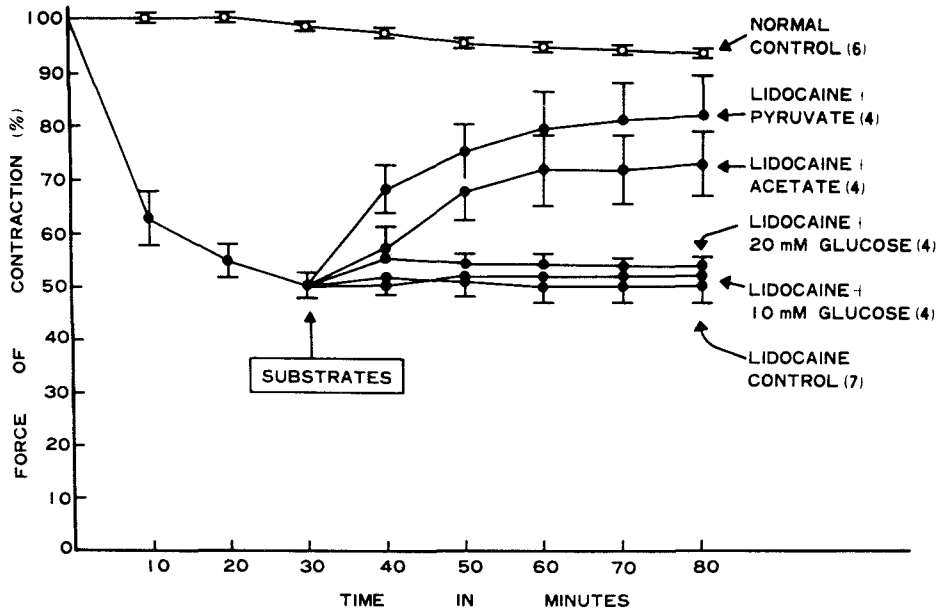

Fig. 1. Effect of substrates on contractility of isolated rat atria depressed with lidocaine (0.1 mM). Substrates were added 30 minutes after the addition of lidocaine. In this and subsequent figures lidocaine was added at zero time (following a 60 minutes equilibration period in the normal Krebs-Ringer bicarbonate glucose medium). Vertical bars represent ± one standard error of the mean Parentheses represent number of experiments.

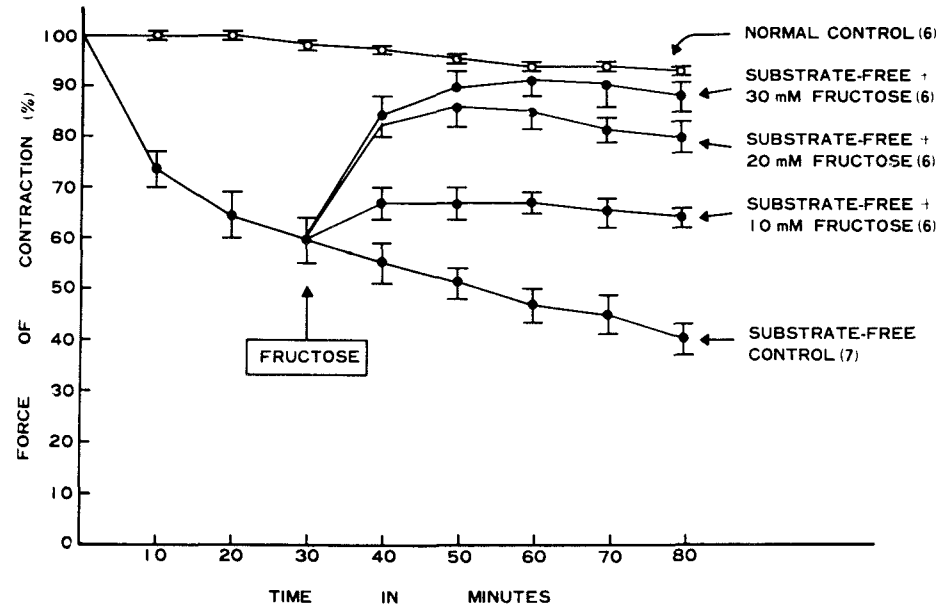

Fig. 2. Effect of fructose on substrate-depleted atria. In this figure zero time is that time following a 60 minutes equilibration of the atria in normal Krebs-Ringer bicarbonate glucose medium. By substrate-depleted atria is menat exposure to substrate-free, but otherwise normal, medium, Fructose was added 30 minutes after exposure to substrate-free medium. Vertical bars indicate standard error of the mean. Parentheses represent number of experiments.

mpt and sustained increase in the force of contraction of atria despite the presence of lidocaine in the bathing medium (Fig. 3).

The effect was similar to that seen with pyruvate and acetate, but not glucose, on lidocaine-depressed atria (Fig. 1), and on general anesthetic halothane-depressed atria (Ko and Paradise, 1969a).

This antagonism of lidocaine depression by fructose but not glucose, along with the evidence pointing to the utilization of fructose via the phosphofructokinase step, suggests that the mechanism of the negative inotropic action of lidocaine in these atria may be blockade of the uptake or utilization of glucose prior to phosphofructokinase step.

Effect of fructose on normal atria

Addition of 30 mM fructose to atria bathed in the normal Krebs-Ringer bicarbonate glucose medium resulted in no demonstrable change in cardiac contractility (Fig. 3).

The results confirm similar observations by Gimeno et al. (1969), and emphasize the importance of lidocaine for the positive inotropic effect of fructose.

Effect of omission of calcium from the medium on atrial contractility

Experiments to further clarify the positive inotropic effect of fructose on the lidocaine-depressed atria were designed employing different experimental conditions. It is well established that calcium ions play an important roles for the contractile activity of the myocardium (Ko and Pardise, 1970c). Therefore, in order to determine first, if or what concentration of calcium removal from the normal medium decline 50% depression of the fore of contraction of normal atria, the low-calcium medium, prepared by omitting a ½ or ¾ amount of calcium chloride from the normal Krebs-Ringer bicarbonate

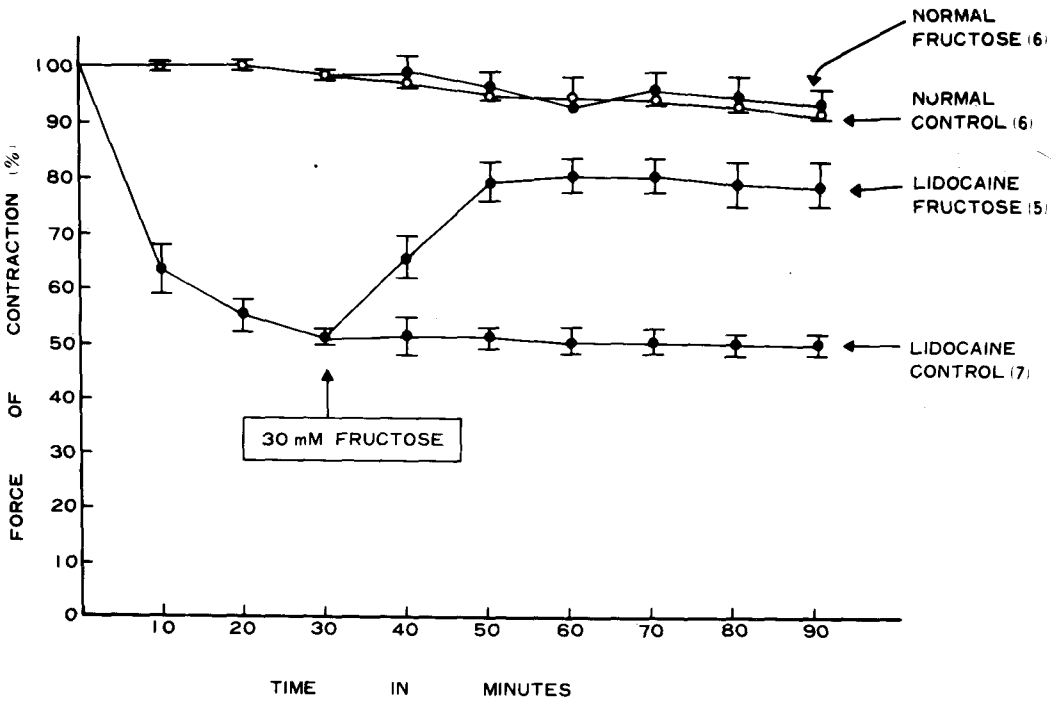

Fig. 3. Effect of fructose (30 mM) on contractility of isolated rat atria depressed with lidocaine (0.1mM). 30 mM fructose was added 30 minutes after administration of 0.1 mM lidocaine. Vertical bars represent standard error of the mean. Parentheses represent number of experiments.

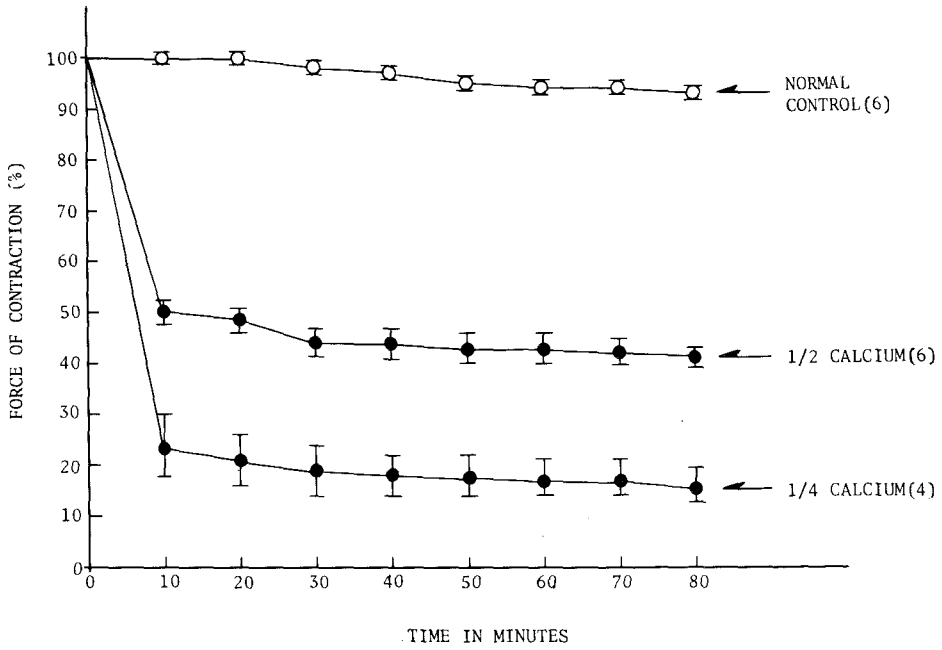

Fig. 4. Effect of calcium removal from the normal Krebs-Ringer bicarbonate glucose medium. Zero time represents one hour in the normal medium. At zero time the normal Krebs-Ringer bicarbonate medium was changed to low-calcium (½ or ¼) medium. Vertical bars represent standard error of the mean. Parentheses represent number of experiments.

glucose medium, was used rather than lidocaine to depress the atrial contractility.

Immediately after a 60 minutes of equilibration period in the Krebs-Ringer bicarbonate glucose medium, the normal medium was replaced with low-calium medium (½ or ¼ calcium medium).

It is evident from the Fig. 4 that the removal of half the calcium from the normal medium (½ calcium medium) resulted in an approximately 50% decrease in atrial contractility similar to that seen with 0.1 mM lidocaine.

Thus, in the experiments with low-calcium medium, ½ calcium medium was chosen for the fructose study because it produced about the same degree of contractile depression of atria as that seen with lidocaine or general anesthetics (Ko and Paradise, 1969a; Ko and Yoon, 1980; Ko and Paik, 1983) and many other cardiac depressants (Ko et al. 1969b; Ko and Paradise, 1970b; 1970c; 1970d).

Effect of fructose on atria depressed with low-calcium medium

Atria were suspended in the normal Krebs-Ringer bicarbonate glucose medium, and allowed for an one-hour equilibration period before the experiments were begun. Immediately after the equilibration period, the normal medium was changed to the low-calcium (½) medium. After 30 minutes incubation of atria in this low calcium medium, fructose at a concentration of 30 mM in effective concentration to increase the lidocaine-depressed atria, was added to the bathing medium in which the atria were beating (at the time the atrial contractility was depressed about 50% in the low-calcium medium).

The results are shown in Fig. 5. It is evident from the Fig. 5 that fructose did not produce any significant effect on the contractility of atria depressed with low-calcium medium. These results differ from those seen with lidocaine experiments in that pyruvate and acetate were effective in increasing the contractility of lidocaine depressed atria (Fig. 1).

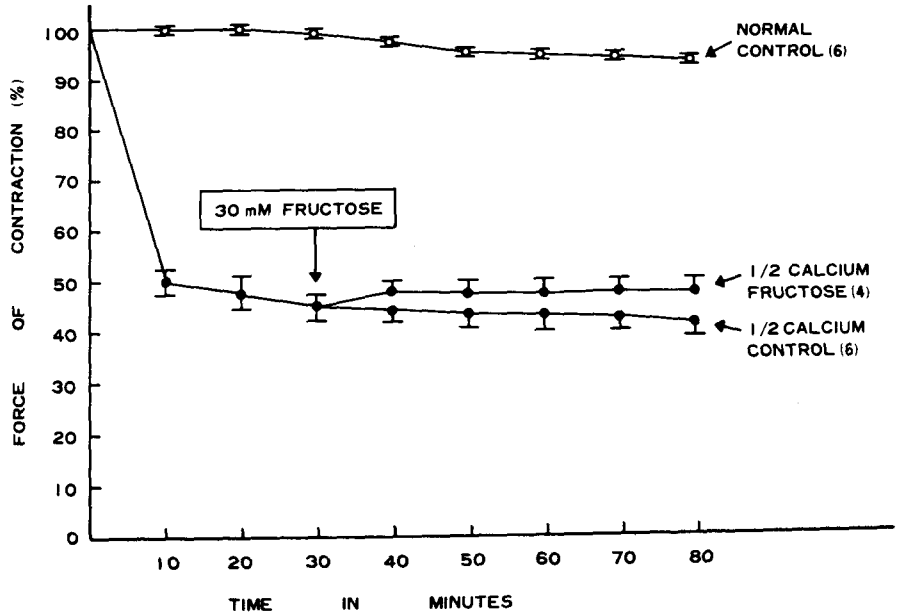

Fig. 5. Effect of fructose (30 mM) on contractility of isolated rat atria depressed with low-calcium medium (½ amount of calcium). 30 mM fructose was added to low-calcium-depressed atria at the 30 minutes period. Control represents atria incubated with ½ calcium medium for 80 minutes without addition of frcutose. Vertical bars represent standard error of the mean. Parentheses represent number of experiments.

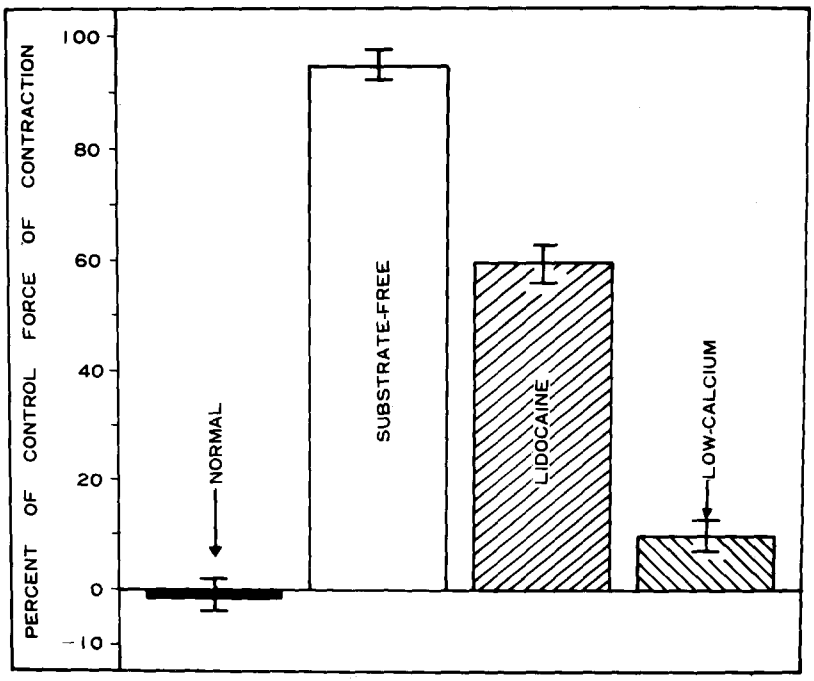

Fig. 6. Ability of fructose to produce a maximal increase in the force of contraction of normal atria, and atria depressed by lidocaine (0.1 mM), low-calcium (1/2) media, or substrate-free medium. Bars indicate the mean increase in force of contraction produced by fructose (30 mM) 30 minutes after its addition as a function of its untreated control point. Vertical lines represent standard error.

DISUCSSION

It was found that pyruvate and acetate partially effective in restoring the contractile activity of isolated rat atria in the hypodynamic state induced by lidocaine (Fig. 1), whereas additional glucose at any concentraction tested had no significant effect on the contractility of lidocaine-depressed atria (Lim and Kim 1984).

However, it has been reported in the literatures that the additional glucose produced that dose-dependent increase in the force of contraction of normal atria (Ko and Paradise 1973b). Whereas the addition of pyruvate produced only as slight increase in the contractile force of normal atria, or even acetate produced slight decrease in the contractile activity of the normal atria (Ko and Paradise, 1969).

From the results with the previous experiments, it is concluded that at least part of negative inotropic action of lidocaine is the result of inhibition of glucose uptake or blockade of glucose utilization in the glycolytic pathway of the heart.

The present studies represent an attempt to localize further the site of lidocaine action in the glycolytic sequence by using an another metabolic substrate fructose. We found a few references with respect to the utilization of fructose by the heart, and those indicated that fructose was a poorly utilized substrate compared with glucose (Gimeno *et al.*, 1969; Opie *et al.*, 1962).

Therefore, we first determined dose-response curves in the substrate-depleted heart to determine if, and at what concentrations, fructose could serve as a source of fuel for the contractile process.

It was found that the maximal effective concentration of fructose on the contractility of substrate-depleted atria was 30 mM (Fig. 2).

Ko and Paradise have established the effect of fructose on heart metabolism, and demonstrated as followings: 1) fructose can serve as a source of fuel for the contraction of isolated rat atria; 2) metabolism of fructose occurs via the phosphofructokinase step in the heart; 3) fructose partially restores the contractility of atria depressed by the general anesthetic halothane (Paradise and Ko, 1970).

Fructose, when used in high concentration (30 mM), has been shown to serve as an exallent substrate for the maintenance of contractility by the isolated rat atria (Fig. 2), despite the developed tension of substrate-depleted atria progressively depressed. Opie et al., (1962) found fructose (5 mM) to be taken up and metabolized to CO_2 less than 1/5 as rapidly as glucose (5 mM). Gimeno et al. (1969) demonstrated that fructose, at concentration of 5.5 or 11 mM, is utilized for contractility but not nearly as efficiently as the corresponding concentration of glucose. Thus the uptake of fructose or its conversion to fructose-6-phosphate may be rate-limiting, higher concentrations of fructose than glucose being necessary for similar effects.

The positive inotropic effect of fructose on lidocaine-depressed, but not normal atria (Fig. 3), along with the previous experiments showing a lack of positive inotropic effect of additional glucose in lidocaine-depressed atria, suggest that the negative inotropic effect of lidocaine is at least partly the reuslt of an interference with glucose uptake or glucose metabolism prior to the enzyme step of phosphofructokinase in the heart.

The effect of fructose on lidocaine-depressed atria was confirmed in different experimental conditions by using low-calcium (1/2) medium (Fig. 5) in which the contractility of isolated rat atria was depressed about 50% similar depression to those of 0.1 mM lidocaine. The data obtained from the experiments with low-calcium medium, the additon of fructose to atria depressed with the low-calcium did not produce any significant effect to increase the force of contraction of low-calcium-depressed atria (Fig. 5).

From this study, the abilities of fructose to produce a maximal increase in the force of contraction of atria in various experimental conditions were compared in Fig. 6. Fructose produced marked ability to increase in the force of contraction of atria depressed with substrate-free medium, and atria depressed

with lidocaine, but did not produce appreciable on the normal atria, or atria depressed with low-calcium medium (Fig. 6).

These results may be further consistent with the hypothesis obtained from the present investigation on the cardiac depressent action of lidocaine.

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=국문초록=

Lidocaine에 의해 억제된 심근수축력에 대한 Fructose의 영향

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Lidocaine의 심근수축력 억제작용에 관한 기전연구 일환으로 lidocaine에 의해 수축력이 감소된 흰쥐 적출심방에 대한 fructose의 효과를 검토하였다.

Fructose는 기질제거에 의해 감소된 적출심방의 수축력을 현저히 증가시켰으며, 30mM에서 최대 증가효과를 나타냈다. Krebs-Ringer glucose 용액에 현수한 적출심방의 수축력은 0.1mM lidocaine에 의해 약 50%의 감소를 나타냈으며, 30 mM fructose의 투여는 이 감소된 수축력을 현저히 증가시켰다. Lidocaine 억제심방에 대한 fructose의 실험 성적은 pyruvate나 acetate에서 얻은 실험성적과 유사하였다.

그러나 같은 농도의 fructose는 저 calcium(1/2)농도의 Krebs-Ringer glucose medium에서 감소(약 50% 감소)된 적출심방의 수축력을 증가시키지 못하였다.

이상의 결과에서 licocaine은 심근내의 포도당 대사를 해당과정에서 억제한다는 가능성을 재확인하고 있으며, 나아가서 lidocaine은 해당과정의 phosphofructokinase step 이전의 초기단계에서 억제하고 있을 가능성을 시사하고 있다.